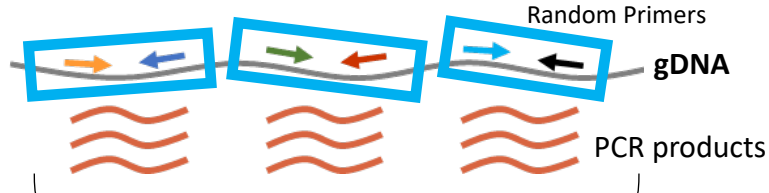


(A)

1) Creating a sequence library

A high concentration of random primers is used in PCR to amplify DNA fragments from the entire genome



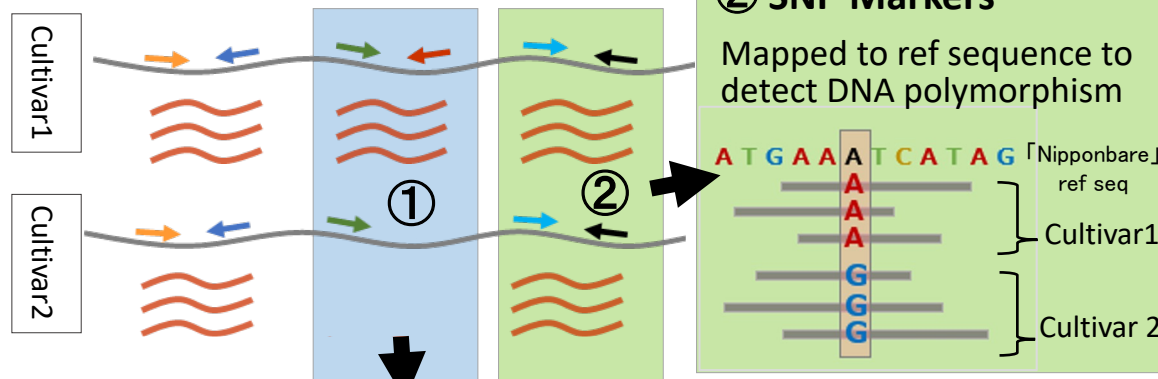
2) Sequence of PCR products using NGS platforms

About 1-fold the amount of rice genome was sequenced using HiSeq2500 NGS-platform (Illumina, Inc., San Diego, CA, USA)



HiSeq2500 NGS-platform

3) Detection of DNA polymorphism

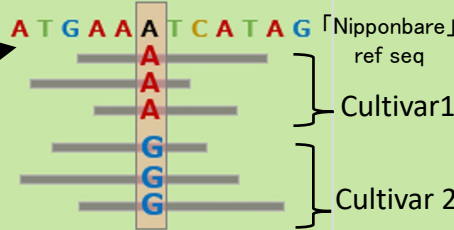


① Amplicon markers

DNA polymorphism judged based on the presence/absence of specific amplification of sequences only in one parent variety.

② SNP Markers

Mapped to ref sequence to detect DNA polymorphism



① Amplicon markers:

▷ "GRAS-Di release version" [Toyota Motor Development]

② SNP markers :

▷ Mapping to the Nipponbare reference seq (IRGSP-1.0) [bowtie2 (ver.2.3.3.1)]

▷ Detection of DNA polymorphism [samtools (ver.1.6) • bcftools (ver.1.6)]

▷ Genotype estimation [beagle (ver.4.0)]

▷ Kosambi's mapping function was used to convert recombination frequency to a genetic map distance (Kosambi, 1943). The R package R/qtl was used to display the linkage maps.

(B)

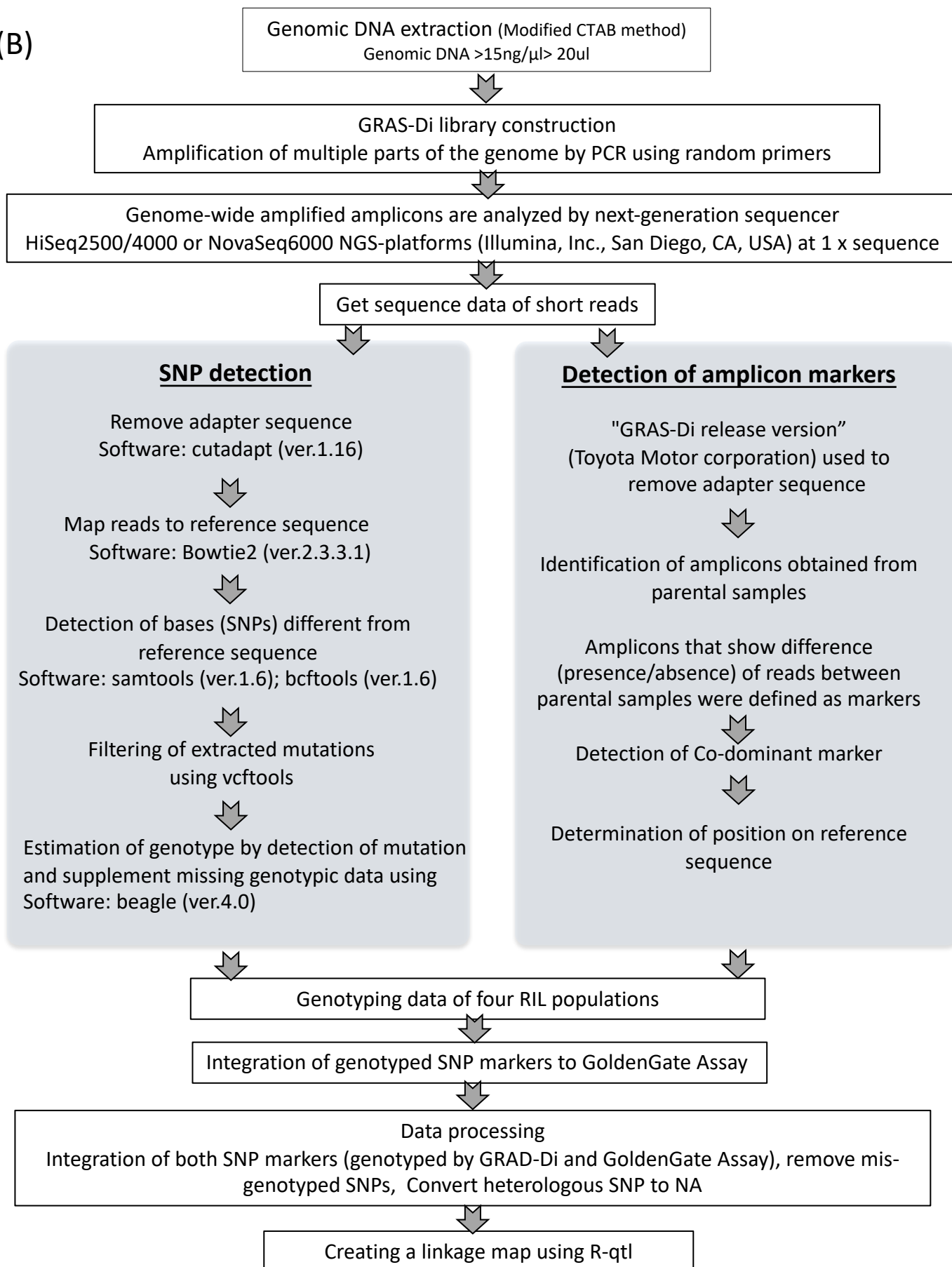


Figure S1. (A) Scheme summarizing the main steps for GRAS-Di library construction in this work . (B) Flow chart of main steps adopted for genotyping analysis and linkage mapping

Table S1. Primer sequences for the first PCR

Primer Name	Primer Sequence (5'-3')
NE10_301	TAAGAGACAGAAA
NE10_302	TAAGAGACAGAAC
NE10_303	TAAGAGACAGAAG
NE10_304	TAAGAGACAGAAT
NE10_305	TAAGAGACAGACA
NE10_306	TAAGAGACAGACC
NE10_307	TAAGAGACAGACG
NE10_308	TAAGAGACAGACT
NE10_309	TAAGAGACAGAGA
NE10_310	TAAGAGACAGAGC
NE10_311	TAAGAGACAGAGG
NE10_312	TAAGAGACAGAGT
NE10_313	TAAGAGACAGATA
NE10_314	TAAGAGACAGATC
NE10_315	TAAGAGACAGATG
NE10_316	TAAGAGACAGATT
NE10_317	TAAGAGACAGCAA
NE10_318	TAAGAGACAGCAC
NE10_319	TAAGAGACAGCAG
NE10_320	TAAGAGACAGCAT
NE10_321	TAAGAGACAGCCA
NE10_322	TAAGAGACAGCCC
NE10_323	TAAGAGACAGCCG
NE10_324	TAAGAGACAGCCT
NE10_325	TAAGAGACAGCGA
NE10_326	TAAGAGACAGCGC
NE10_327	TAAGAGACAGCGG
NE10_328	TAAGAGACAGCGT
NE10_329	TAAGAGACAGCTA
NE10_330	TAAGAGACAGCTC
NE10_331	TAAGAGACAGCTG
NE10_332	TAAGAGACAGCTT
NE10_333	TAAGAGACAGGAA
NE10_334	TAAGAGACAGGAC
NE10_335	TAAGAGACAGGAG
NE10_336	TAAGAGACAGGAT
NE10_337	TAAGAGACAGGCA
NE10_338	TAAGAGACAGGCC
NE10_339	TAAGAGACAGGCG
NE10_340	TAAGAGACAGGCT
NE10_341	TAAGAGACAGGGA
NE10_342	TAAGAGACAGGGC
NE10_343	TAAGAGACAGGGG
NE10_344	TAAGAGACAGGGT
NE10_345	TAAGAGACAGGTA
NE10_346	TAAGAGACAGGTC
NE10_347	TAAGAGACAGGTG
NE10_348	TAAGAGACAGGTT
NE10_349	TAAGAGACAGTAA
NE10_350	TAAGAGACAGTAC
NE10_351	TAAGAGACAGTAG
NE10_352	TAAGAGACAGTAT
NE10_353	TAAGAGACAGTCA
NE10_354	TAAGAGACAGTCC
NE10_355	TAAGAGACAGTCG
NE10_356	TAAGAGACAGTCT
NE10_357	TAAGAGACAGTGA
NE10_359	TAAGAGACAGTGG
NE10_360	TAAGAGACAGTGT
NE10_361	TAAGAGACAGTTA
NE10_362	TAAGAGACAGTTC
NE10_363	TAAGAGACAGTTG
NE10_364	TAAGAGACAGTTT

Table S2. Primer sequences for the second PCR

GRAS-Di library construct (Illumina Nextera adapter sequence used)										
(P5 side) 5'-AATGATACGGCACCACCGAGATCTACAC[P5 index sequence(below)]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG[Insert]CTGTCTCTTATACATCTCCGAGCCACGAGAC[P7 index sequence(below)]ATCTCGTATGCCGCTCTTCTGTTG-3' (P7 side)										
P5 Index Name	Index Primer Sequence (5'-3')				Index Sequence	P7 Index Name	Index Primer Sequence (5'-3')			Index Sequence
P5_0001	AATGATACGGCACCACCGAGATCTACAC CGCGCAGAT CGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CGCGCAGA	P7_0001	CAAGCAGAAGACGGCATAACGAGAT TCGTACAG AGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCTGACGA
P5_0002	AATGATACGGCACCACCGAGATCTACAC CGTCAGCAT CTCGCGCAGCGTCAGATGTGTATAAGAGACAG				CGTCAGCA	P7_0002	CAAGCAGAAGACGGCATAACGAGAT CGCTACGGC GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CGCTACGG
P5_0003	AATGATACGGCACCACCGAGATCTACAC AGCGTCGAT CGTCGGCAGCGTCAGATGTGTATAAGAGACAG				AGCGTCGA	P7_0003	CAAGCAGAAGACGGCATAACGAGAT TCTCAGTA GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TACTGAGA
P5_0004	AATGATACGGCACCACCGAGATCTACAC CATATGTAT CTCGCGCAGCGTCAGATGTGTATAAGAGACAG				CATATGTA	P7_0005	CAAGCAGAAGACGGCATAACGAGAT ACGAGCAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTGCTCGT
P5_0005	AATGATACGGCACCACCGAGATCTACAC CAGACAGAT CGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CAGACAGA	P7_0006	CAAGCAGAAGACGGCATAACGAGAT ATACGTGAGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCACGTAT
P5_0007	AATGATACGGCACCACCGAGATCTACAC ACTCACAT CTCGCGCAGCGTCAGATGTGTATAAGAGACAG				ACTCACAT	P7_0007	CAAGCAGAAGACGGCATAACGAGAT GCTACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCTAGAC
P5_0008	AATGATACGGCACCACCGAGATCTACAC CGCTCTAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GCTCTAGT	P7_0009	CAAGCAGAAGACGGCATAACGAGAT CTCACAG AGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCGTGTAG
P5_0009	AATGATACGGCACCACCGAGATCTACAC CGCCTCAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GCGCTCAT	P7_0010	CAAGCAGAAGACGGCATAACGAGAT AGACATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATGTGCT
P5_0010	AATGATACGGCACCACCGAGATCTACAC CAGAGTAGAT CGTCGGCAGCGTCAGATGTGTATAAGAGACAG				AGAGTAGA	P7_0012	CAAGCAGAAGACGGCATAACGAGAT TGATAGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCTATCA
P5_0011	AATGATACGGCACCACCGAGATCTACAC TATGAGCAT CGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TATGAGCA	P7_0013	CAAGCAGAAGACGGCATAACGAGAT GACGACTG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CAGTCGTC
P5_0012	AATGATACGGCACCACCGAGATCTACAC CACTGATTCT CGCGCAGCGTCAGATGTGTATAAGAGACAG				TCACTGAT	P7_0014	CAAGCAGAAGACGGCATAACGAGAT TGTGCTAG TCGTCGGGCTCGGAGATGTGTATAAGAGACAG			TGAGCACA
P5_0013	AATGATACGGCACCACCGAGATCTACAC CTGACAGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CTGACAGT	P7_0018	CAAGCAGAAGACGGCATAACGAGAT CTGTCGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCGTGAGA
P5_0014	AATGATACGGCACCACCGAGATCTACAC CGCGAGCTAT CTCGTCGAGCGTCAGATGTGTATAAGAGACAG				GCGAGCTA	P7_0002A	CAAGCAGAAGACGGCATAACGAGAT TGATATAT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			GATATCAT
P5_0015	AATGATACGGCACCACCGAGATCTACAC CATATCTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CATATCGT	P7_0003A	CAAGCAGAAGACGGCATAACGAGAT GTATCTAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTAGATAC
P5_0016	AATGATACGGCACCACCGAGATCTACAC TCAGCAGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TCAGCAGT	P7_0005A	CAAGCAGAAGACGGCATAACGAGAT ATCTAGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCTAGAT
P5_0017	AATGATACGGCACCACCGAGATCTACAC CAGATGTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GACATGTG	P7_0006A	CAAGCAGAAGACGGCATAACGAGAT GCTCTATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATAGACG
P5_0018	AATGATACGGCACCACCGAGATCTACAC TGTATGAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TGTATGAG	P7_0007A	CAAGCAGAAGACGGCATAACGAGAT AGTGTAGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TACATACT
P5_0019	AATGATACGGCACCACCGAGATCTACAC CAGATCAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CAGATCAG	P7_0008A	CAAGCAGAAGACGGCATAACGAGAT GACTACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTGTAGTC
P5_0003A	AATGATACGGCACCACCGAGATCTACAC TGCTACATG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TGCTATAG	P7_0009A	CAAGCAGAAGACGGCATAACGAGAT ATAGCTGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CAGCTCAT
P5_0006A	AATGATACGGCACCACCGAGATCTACAC CTAGCTCT CTCGCGCAGCGTCAGATGTGTATAAGAGACAG				TGAGTCTA	P7_0010A	CAAGCAGAAGACGGCATAACGAGAT ATAGACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			GTCTATCT
P5_0007A	AATGATACGGCACCACCGAGATCTACAC CAGTACAGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GAGTACAG	P7_0011A	CAAGCAGAAGACGGCATAACGAGAT CTGTCGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTGTATAG
P5_0008A	AATGATACGGCACCACCGAGATCTACAC TATACTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TATACTGA	P7_0012A	CAAGCAGAAGACGGCATAACGAGAT TAGATGTAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TACTACTA
P5_0010A	AATGATACGGCACCACCGAGATCTACAC CTAGTACT CTCGCGCAGCGTCAGATGTGTATAAGAGACAG				CATGTACA	P7_0013A	CAAGCAGAAGACGGCATAACGAGAT GAGACATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATGTTCT
P5_0011A	AATGATACGGCACCACCGAGATCTACAC TACTCGCT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TACTCGCT	P7_0014A	CAAGCAGAAGACGGCATAACGAGAT AGAGTAGG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCACTCT
P5_0012A	AATGATACGGCACCACCGAGATCTACAC CAGTGTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CAGTGTGT	P7_0016A	CAAGCAGAAGACGGCATAACGAGAT AGACTATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATAGTCT
P5_0013A	AATGATACGGCACCACCGAGATCTACAC TATGTAGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TATGTAGT	P7_0019A	CAAGCAGAAGACGGCATAACGAGAT AGCTGATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCTCAGCT
P5_0014A	AATGATACGGCACCACCGAGATCTACAC CTGAGATG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GTGAGATG	P7_0022A	CAAGCAGAAGACGGCATAACGAGAT TGTATGAGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCATCAT
P5_0015A	AATGATACGGCACCACCGAGATCTACAC TAGACTAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TAGACTAG	P7_0024A	CAAGCAGAAGACGGCATAACGAGAT TCTGATAGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTATCAGA
P5_0016A	AATGATACGGCACCACCGAGATCTACAC GTGTACGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GTGTACGT	P7_0030A	CAAGCAGAAGACGGCATAACGAGAT CGTATGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCATACG
P5_0018A	AATGATACGGCACCACCGAGATCTACAC TGAGCTAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TGAGCTAT	P7_0036A	CAAGCAGAAGACGGCATAACGAGAT GTCATATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATATGAC
P5_0019A	AATGATACGGCACCACCGAGATCTACAC CAGCTGTA TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CAGCTGTA	P7_0040A	CAAGCAGAAGACGGCATAACGAGAT GCAGAGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCTCTGC
P5_0020A	AATGATACGGCACCACCGAGATCTACAC CACGAGAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CACGAGAG	P7_0041A	CAAGCAGAAGACGGCATAACGAGAT GATGAGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCTCATGC
P5_0021A	AATGATACGGCACCACCGAGATCTACAC CAGTGATA TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CAGTGATA	P7_0042A	CAAGCAGAAGACGGCATAACGAGAT GATGTGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTCACATC
P5_0022A	AATGATACGGCACCACCGAGATCTACAC CGCTATAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GCGTATAT	P7_0047A	CAAGCAGAAGACGGCATAACGAGAT CAGTGTAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATCACTG
P5_0023A	AATGATACGGCACCACCGAGATCTACAC GATCAGCA TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GATCAGCA	P7_0048A	CAAGCAGAAGACGGCATAACGAGAT CTCATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTATCAG
P5_0025A	AATGATACGGCACCACCGAGATCTACAC ACACTCAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				ACACTCAG	P7_0050A	CAAGCAGAAGACGGCATAACGAGAT GATGATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTACTATC
P5_0026A	AATGATACGGCACCACCGAGATCTACAC TCATCTAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TCACTATA	P7_0055A	CAAGCAGAAGACGGCATAACGAGAT ACGTACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			GTGTACGT
P5_0029A	AATGATACGGCACCACCGAGATCTACAC CGCAGATAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GCGCATA	P7_0056A	CAAGCAGAAGACGGCATAACGAGAT TACTGATAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCTACGTA
P5_0030A	AATGATACGGCACCACCGAGATCTACAC CTATGTAG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CTATGTAG	P7_0068A	CAAGCAGAAGACGGCATAACGAGAT TGCTACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCGTAGCA
P5_0031A	AATGATACGGCACCACCGAGATCTACAC CTGCTCGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CTGCTCGT	P7_0073A	CAAGCAGAAGACGGCATAACGAGAT ATGACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTGTCAT
P5_0032A	AATGATACGGCACCACCGAGATCTACAC GTAGTGCT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GTAGTGCT	P7_0074A	CAAGCAGAAGACGGCATAACGAGAT TGAGACAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTGTCTCA
P5_0033A	AATGATACGGCACCACCGAGATCTACAC ATGATAGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				ATGATAGT	P7_0078A	CAAGCAGAAGACGGCATAACGAGAT ACTCATGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCATGAGT
P5_0034A	AATGATACGGCACCACCGAGATCTACAC AGCAGACT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				AGCAGACT	P7_0086A	CAAGCAGAAGACGGCATAACGAGAT ACGATGAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TCGTGCTG
P5_0036A	AATGATACGGCACCACCGAGATCTACAC TCGACGTAT CTCGCAGCGTCAGATGTGTATAAGAGACAG				TGCAGCTA	P7_0091A	CAAGCAGAAGACGGCATAACGAGAT GAGTCAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TACGTCGT
P5_0037A	AATGATACGGCACCACCGAGATCTACAC CAGCAGCT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GACAGCGT	P7_0094A	CAAGCAGAAGACGGCATAACGAGAT GACGTCTG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TAGACGTC
P5_0039A	AATGATACGGCACCACCGAGATCTACAC CGCAGTGA TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CGCAGTGA	P7_0095A	CAAGCAGAAGACGGCATAACGAGAT CACGTAGT GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTACAGTG
P5_0040A	AATGATACGGCACCACCGAGATCTACAC CAGATGTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				TCAGATGT	P7_0097A	CAAGCAGAAGACGGCATAACGAGAT GATCATG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TATGATAC
P5_0042A	AATGATACGGCACCACCGAGATCTACAC CGAGACGAT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				CGAGACGA	P7_0100A	CAAGCAGAAGACGGCATAACGAGAT CAGATCAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CTGATCTG
P5_0049A	AATGATACGGCACCACCGAGATCTACAC GTGAGTGT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GTGAGTGT	P7_0102A	CAAGCAGAAGACGGCATAACGAGAT AGCGACG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			CGCTGCTG
P5_0050A	AATGATACGGCACCACCGAGATCTACAC GATGCACT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG				GATGCACT	P7_0103A	CAAGCAGAAGACGGCATAACGAGAT GAGATCAG GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			TGATCTGC

GRAS-Di markers			
	SNP markers	Amplicon markers	Total
RIL71	495	555	1050
RIL98	499	786	1285
RIL16	593	1115	1708
RIL91	635	1069	1704

Figure S2. Total number of markers generated by GRAS-Di

RIL71		GoldenGate			
		A	B	-	total
GRAS-Di SNP+ Amplicon markers	A	11080	18	14	11112
	B	28	10995	46	11069
	-	8	5	606	619
	total	11116	11018	666	22121
corresponding ratio	0.997920528005				

RIL98		GoldenGate			
		A	B	-	total
GRAS-Di SNP+ Amplicon markers	A	7291	11	14	7316
	B	11	7480	3	7494
	-	22	9	423	454
	total	7324	7500	440	14793
corresponding ratio	0.998512810113				

RIL 16		GoldenGate			
		A	B	-	total
GRAS-Di SNP+ Amplicon markers	A	9150	21	41	9212
	B	15	10574	13	10602
	-	11	31	569	611
	total	9176	10626	623	19760
corresponding ratio	0.998178137652				

RIL91		GoldenGate			
		A	B	-	total
GRAS-Di SNP+ Amplicon markers	A	10397	13	32	10442
	B	11	11214	18	11243
	-	6	8	1613	1627
	total	10414	11235	1663	21635
corresponding ratio	0.998890686388				

Figure S3. Example of calculation of correspondence ratio between makers generated by GoldenGate method and markers generated by GRAS-Di technology